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LIPID-LOWERING QUINAZOLINE DERIVATIVE

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LIPID-LOWERING QUINAZOLINE DERIVATIVE

Field of the Invention

This invention relates to novel quinazoline derivatives, for example 4-(3'-bromobenzoyl)-6,7-dimethoxyquinazoline), and methods of use. In particular, the compounds of the invention, when administered to a subject, are effective in reducing blood cholesterol levels in the subject.

Background of the Invention

Atherosclerosis and ischemic heart disease remain the major cause of death of Americans. Elevated serum cholesterol levels present a major risk factor for atherosclerosis and related complications including myocardial infarction, heart failure, and cerebral stroke. Intervention studies performed in middle-aged men demonstrated a marked reduction in the incidence of cardiovascular events after the lowering of elevated total and low-density lipoprotein cholesterol (LDL-C) levels. The Cholesterol and Recurrent Events (CARE) trial and the Scandinavian Simvastatin Survival Study (4S)(1994, *Lancet* 344:1383-1389) have further shown that both women and elderly patients with prior history of ischemic heart disease benefit from cholesterol lowering therapy. (Miettinen et al., 1988, *Arch. Intern. Med.* 148:36-69; Sacks et al., 1996, *New Eng. J. Med.* 335:1001-1009)

The most effective cholesterol lowering drugs are statins, which lower cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme which catalyzes the limiting step in cholesterol biosynthesis (Goldstein et al., 1984, *J. Lipid Res.* 25, 1450-1461). Compared to treatment regimens with other lipid lowering agents, such as the bile acid sequestrants (colestipol and cholestyramine), nicotinic acid, fibric acid (gemfibrozil and clofibrate), probucol, and experimental ACAT inhibitors, statin therapy has been found to bear several favorable features. Statins achieve extensive lowering of LDL-C, leading to an overall reduction in mortality, and are cost effective due to substantial reduction of hospital admissions and rates of coronary intervention. Statins also achieve better compliance than other treatments, as a result of their

once-daily administration and few side effects. Combination of statins with other agents is considered necessary for patients with severe, complex or refractory lipid disorders. Furthermore, large clinical trials have suggested regression of atherosclerotic lesions by aggressive lipid lowering therapy (Schell and Myers, 1997, *Prog. on Cardiovascular Diseases* 39:483-496). To complement the statins and achieve successful reduction of cholesterol in statin-resistant subjects, identification of new lipid lowering agents with a different mechanism of action than statins remains a major focal point in contemporary atherosclerosis research. Since the HMG CoA inhibitors are ineffective in the mouse, this animal provides a useful model for screening novel agents capable of lowering cholesterol levels by a different mechanism of action than statins.

There is a need for new lipid lowering agents that are effective to lower total cholesterol and/or LDL-C, for the treatment of high cholesterol and other lipid disorders including those which are severe, complex, and/or refractory to current treatments.

Summary of the Invention

Quinazoline compounds having a carbonyl substitution (carbonyl-Q) as described below and exemplified by 4-(3'-bromobenzoyl)-6,7-dimethoxyquinazoline (WHI-P164) have now been identified as a new class of potent cholesterol lowering agents. As shown in the Examples below, administration of the WHI-P164 reduces total cholesterol levels in hypercholesterolemic C57Bl/6 mice on a high calorie diet by 23% and LDL-C by 45%. WHI-P164 also reduced total cholesterol levels and β-VLDL/LDL-cholesterol levels of hypercholesterolemic apolipoprotein E deficient mice (*apo* $e^{-/-}$) by 41% and 63%, respectively.

The present invention provides potent cholesterol-lowering agents, quinazoline compounds having a carbonyl group, (carbonyl-Q). An exemplary compound of the invention is 4-(3'-bromobenzoyl)-6,7-dimethoxyquinazoline).

The novel cholesterol lowering agents of the invention may be formulated by means known in the art for delivery to targeted areas of the body, including blood and/or gut, for example, by choice of carrier, mode of administration, or by conjugating carbonyl-Q with a specific targeting moiety, such as an antibody or ligand which binds a specific antigen or ligand receptor in the target tissue. Formulations useful for therapeutic reduction of cholesterol include injectable compositions, oral compositions, depot formulations, and the like containing an effective cholesterol-lowering amount of a carbonyl-Q compound of the invention, such as 4-(3'-bromobenzoyl)-6,7-dimethoxyquinazoline.

In the methods of the invention, carbonyl-Q cholesterol lowering agents such as 4-(3'-bromobenzoyl)-6,7-dimethoxyquinazoline are administered to a subject in order to modulate lipids in the blood, and particularly to lower blood cholesterol.

The above summary of the present invention is not intended to describe each disclosed embodiment or every implementation of the present invention. The examples and the detailed description which follow more particularly exemplify these embodiments.

Brief Description of the Drawings

Figure 1 is a graph showing the reduction of VLDL-C by WHI-P164 treatment in the C57B1/6 hypercholesterolemia model. Cholesterol profiles of C57B1/6 mice fed with a cocoa butter diet and 0 mg/kg/day (open circles); 1.6 mg/kg/day (open squares); or 16 mg/kg/day (closed circles) (n=4) are shown.

Figure 2 is a graph showing reduction of VLDL-C by WHI-P164 treatment in *apo-e*$^{-/-}$ mice fed on rodent chow and treated with WHI-P164 at 8 mg/kg/day for 7 days (closed circles, n=8) or with vehicle alone (open circles, n=4).

Figure 3 is a graph showing reduction of VLDL-C by WHI-P164 treatment in *apo-e*$^{-/-}$ mice fed with a high fat, high cholesterol diet. Cholesterol profiles of *apo-e*$^{-/-}$ mice fed a Western diet and treated with WHI-P164 at 40 mg/kg/day for 7 days (closed circles, n=2) or with vehicle alone (open circles, n=3) are shown.

Figures 4A-4D are photographs showing reduction of hepatic triglyceride synthesis by WHI-P164 treatment. Livers of *apo-e*$^{-/-}$ mice fed a Western diet and treated with vehicle alone (Figures 4A and 4C) or with 1 mg/day of WHI-P164 for one week (Figures 4B and 4D) were analyzed using Oil Red O staining (Figures 4A and 4B) and Haemotoxylin and Eosin tissue section staining (Figures 4C and 4D).

Figure 5 is a graph showing inhibition of postprandial triglyceride accumulation by WHI-P164 treatment. C57B1/6 mice were treated with WHI-P164 at 40 mg/kg/day for 7 days, and fasted 36 hours with continuing treatment. Food was readministered at 0, 1, 3, 6, and 9 hours. WHI-P164 treated mice (closed circles) exhibited much reduced postprandial triglyceride accumulation in comparison to vehicle treated mice (open circles).

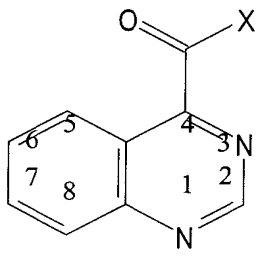
Figures 6A-D demonstrate WHI-P164 induced regression of pre-existing atherosclerotic lesions in hypercholesterolemic *apo-e*$^{-/-}$ mice. Aortas from untreated (Figure 6A), vehicle treated (Figure 6B), and WHI-P164 treated (Figure 6C) animals were stained with Sudan IV to show the fatty streaks. Aortas of untreated control mice were obtained at 7 months to reflect the pretreatment condition of the test groups. Aortas of vehicle treated or WHI-P164 treated test mice were obtained at 8 months following a one month treatment program. Statistical comparisons indicate **p=0.002 for untreated controls versus WHI-P164 treated test mice and p=0.007 for vehicle controls versus WHI-P164 treated mice.

Detailed Description of the Preferred Embodiments

The invention includes a cholesterol lowering compound comprising a carbonyl substituted quinazoline (carbonyl-Q) exemplified by 4-(3'-bromobenzoyl)-6,7-dimethoxyquinazoline (WHI-P164) and a method for lowering blood cholesterol by administration of a carbonyl-Q.

Cholesterol-Lowering Compounds of the Invention

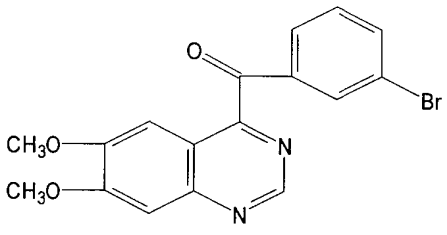
The cholesterol-lowering compounds of the invention include carbonyl-substituted quinazolines (carbonyl-Q) having the following general structural formula:



(carbonyl-Q)

where X is an alkyl (straight chain, branched, or cyclic) or is an aromatic ring structure, such as phenyl, napthyl, and the like. X may be substituted, for example, with a halogen, OH, SH, alkyl, alkoxy, acyloxy, NH_2, and the like. Carbonyl-Q may be substituted, for example, carbon or nitrogen atoms at positions 2, 5, 6, or 8 may have bound thereto halo, H, OH, alkyl, alkoxy, acyloxy, and the like groups.

An exemplary carbonyl-Q compound of the invention is 4-(3'-bromobenzoyl)-6,7-dimethoxyquinazoline (WHI-P164), having the following structure:



(WHI-P164)

The cholesterol-lowering compounds of the invention are formulated into compositions for administration, preferably including a carrier which assists in the administration of the compound for cholesterol-lowering effects. For example, the composition may include a carrier which aids in suspending or solubilizing the compound of the invention or to improve the flavor or texture of the composition in

foodstuffs or beverages for oral delivery to the gut. Alternatively, a composition containing the compound of the invention may include an isotonic carrier to facilitate delivery by injection, agents to prolong the half-life of the compound, and the like.

Multiple delivery systems are known for delivery of compounds to the bloodstream and gut of an animal. Preferably, the compound of the invention is administered to the gut, most preferably by oral delivery in a foodstuff composition, such as a dietary supplement or a staple foodstuff supplemented with the composition of the invention.

Mode of Administration

The cholesterol-lowering compounds of the invention can be formulated as pharmaceutical compositions, nutritional supplements, or as additives in foodstuffs and administered to a mammalian host, including a human in a variety of forms adapted for administration of a quinazoline compound. Preferred administration routes include intravenous, intramuscular, and subcutaneous injection. Most preferred is oral administration.

Solutions or suspensions of the cholesterol-lowering composition can be prepared in water, isotonic saline (PBS) and may preferably be mixed with a non-toxic surfactant. Dispersions may also be prepared in glycerol, vegetable oils, and the like. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

The dose of the composition to be administered can vary widely in accordance with the age, size, and condition of the subject to be treated. Useful dosages of the composition are those which will deliver about 0.1 to about 500 mg/kg body weight/day, and preferably deliver about 0.5 to about 10 mg/kg body weight/day. The amount of the compound needed in the composition can vary with the mode of administration, e.g., by injection or by oral administration, to account for variance in the metabolism of the compound and of the composition.

While the invention is amenable to various modifications and alternative forms, specific embodiments of the invention are shown by way of the Examples and will be described in detail. It should be understood, however, that the intention is not to limit the invention to the particular embodiments described. On the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention.

EXAMPLES

The invention may be better understood by reference to the following Examples, that are not intended to limit the invention in any way.

Example 1

Animal Models of Hypercholesterolemia and Analytical Methods

Three- to four-week old C57Bl/6 male mice (Taconic, Germantown, NY, USA) were kept in micro-isolator cages on a 12-hour day/night cycle and fed the Paigen's cocoa butter diet (15.8% fat, 1.25% cholesterol, and 0.5% sodium cholate) (Harlan Teklad, Madison, WI, USA) for two weeks prior to initiation of lipid lowering therapy. Paigen's diet is described in Nishina et al., 1993, *J.Lipid Res.* 34: 1413-1422.

Three- to four-week old apolipoprotein E deficient (*apo* $E^{-/-}$) mice were the progeny of breeding pairs of apo E knockout (apo EKO) mice obtained from Jackson Labs. (Bar Harbor, Maine, USA). Mice were fed either regular rodent chow (5% fat and 0% cholesterol) (Teklad-LM 485, Harlan Teklad, Madison, WI, USA), the Paigen's cocoa butter diet, or the Western diet (21.22% fat and 0.2% cholesterol) (Harlan Teklad, Madison, WI, USA) for two weeks prior to treatment.

Animals were treated with test compounds by intraperitoneal injection daily for 7 days. A range of doses was administered. Control animals received vehicle alone. The effect of quinazoline derivatives on total cholesterol, LDL, HDL, liver

enzymes, and glucose was analyzed. The mice tissues were also examined histologically.

Lipid determinations:

Serum total cholesterol was measured enzymatically using the cholesterol kit from Sigma Chemical Co. (St. Louis, MO, USA). For liver tissue lipid measurements, lipids were extracted from homogenized hepatic tissues using the method of Bligh and Dyer (Bligh, et al., *Can. J. Biochem. Phys.* 1959, 37:911-917). One gram of liver tissue was homogenized in 1 ml of distilled water and extracted with 4 ml of chloroform/methanol (1:1, v/v). The chloroform phase containing the lipids was evaporated under a stream of nitrogen, the lipids dissolved in 100 μL of isopropanol, and subjected to cholesterol determination as described above. Triglyceride (TG) contents were determined using the TG kit from Sigma Chemical Co.

Serum cholesterol profiles were examined by Fast Protein Liquid Chromatography (FPLC) using 100 μl of serum per run. The serum was passed over two Superose™ 6 HR 10/30 connected in tandem equilibrated in phosphate buffered saline (PBS), using the FPLC system from Pharmacia Biotech (Piscataway, NJ, USA) consisting of a controller LCC-501 plus connected to a UV detector (UV-MII), two P500 pumps, and a Frac-100 fraction collector. The FPLC was remotely controlled and operated by the FPLC director program operated on an IBM computer. The system was operated at a flow rate of 0.5 ml/minute and fractions were collected at 0.5 ml/fraction. Cholesterol concentration of each fraction was determined as described above and plotted against the fraction numbers to obtain the serum cholesterol profile.

To determine the serum concentrations of LDL/β-VLDL and HDL, the sums of the LDL/β-VLDL peak (fractions 29-35) and the HDL peak (fractions 53-63) were divided by 0.1. To obtain the IC_{50} and the minimum β-VLDL and HDL concentrations, the concentrations of β-VLDL and HDL were plotted against the dosage. Best fitted exponential decay curves were obtained using the equation: Concentration of HDL or LDL/β-VLDL= $Aexp(-BX) + E$, where A+E equals the

concentration of HDL or LDL/β-VLDL at the dosage of 0 μg/day and E equals the mathematical minimum concentration of HDL or β-VLDL, and B equals $0.69/IC_{50}$. Best fitted curves were obtained using the Graphpad Inplot program, Graphpad Software, San Diego, CA, USA.

Determination of *In Vivo* Hepatic VLDL-TG Production Using Triton WR1339

C57Bl/6 and apoE-deficient mice were treated with intraperitoneal injections of 50 μl vehicle or 40 mg/kg WHI-P164 in 50 μl vehicle for 7 consecutive days, followed by a 36 hour fast to shut down intestinal chylomicron synthesis. Subsequently, mice were injected intravenously with 500 mg/kg Triton WR1339 (Sigma Chemical Co.) in 0.9% NaCl to completely inhibit their plasma VLDL clearance, as previously reported (Pasternali et al., 1996, *Ann. Intern. Med.* 125:529-540; Aalto-Setala et al., 1992, *J. Clin. Invest.* 90:1889-1900). Blood samples were taken at 0 and 4 hours after Triton WR1339 injection and plasma TG levels were measured enzymatically using a commercially available enzymatic kit (Sigma Chemical Co.). Alternatively, food was returned to the mice after a 36 hour fast and blood samples were collected at 0, 1, 3, 6, and 9 hours after returning the food. Accumulation of chylomicron-TG was determined as accumulation of plasma TG.

Blood Chemistry

Serum levels alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine, creatinine phosphokinase (CPK), and glucose were determined by using a Synchron CX5 Clinical System (Beckman Instruments, Inc., Fullerton, CA) following manufacturer's instructions. For the glucose tolerance test, blood glucose levels were determined using the portable One Touch Profile glucose meter (Lifescan, Milpitas, CA, USA).

Histopathology

At the end of the experiment, the animals were sacrificed and the harvested tissues were fixed in 10% phosphate buffered formalin overnight, embedded, sectioned, and stained by hematoxylin and eosin (H&E). The stained sections were examined for pathological changes. For Oil Red O staining, the liver was excised and snap frozen in liquid nitrogen. Five micrometer thick sections were obtained using the LeicaCM1800 cryostat (Heerbrugg, Switzerland), stained in 0.5% Oil Red O in propylene glycol for 1 hour, destained in 85% propylene glycol for 1 minute, rinsed twice in distilled water, counterstained in Mayer's hematoxylin for a few seconds, rinsed twice in distilled water, and mounted in Crystalmount (Biomedia Corp., Foster City, CA). The Oil Red O stainable material at 400 X magnification was quantitated using the ImagePro plus program (Media Cybernetics, Silver Spring, MD, USA) in conjunction with a 3CCD camera (DAGE-MTI Inc., Michigan City, USA).

Example 2

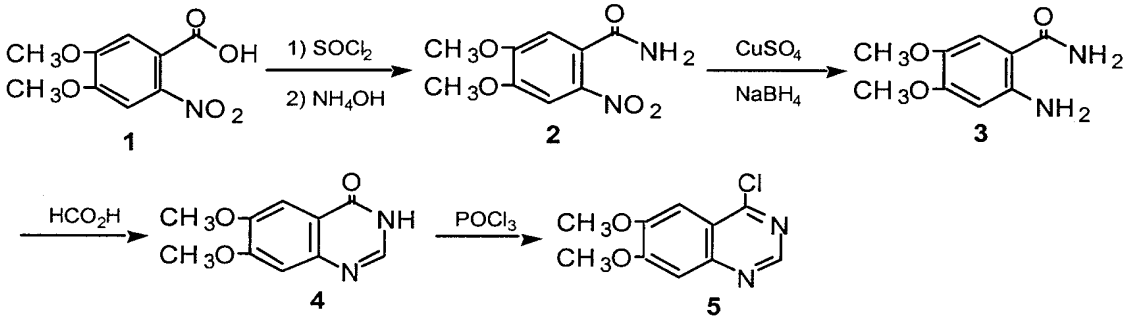
Chemical synthesis and Characterization of Quinazoline Derivatives

The common synthetic intermediate 4-chloro-6,7-dimethoxyquinazoline **5** used for synthesizing all the tested compounds by following literature procedures (Scheme 1). 4,5-dimethoxy-2-nitrobenzoic acid **1** was treated with thionyl chloride, and then reacted with ammonia to give 4,5-dimethoxy-2-nitrobenzamide **2** (Nomoto et al., 1990, *Chem. Pharm. Bull* 38: 1591-1595). The nitro group in compound **2** was reduced with sodium borohydride in the presence of copper sulfate (31) to give 4,5-dimethoxy-2-aminobenzamide **3**, which was cyclized by refluxing with formic acid to give 6,7-dimethoxyquinazoline-4(3H)-one **4**. The quinazolinone **4** was refluxed with phosphorus oxytrichloride to provide the key starting material **5** with good yield.

The lead compound 4-(3'-bromobenzoyl)-6,7-dimethoxyquinazoline WHI-P164 was synthesized by reacting 4-chloro-6,7-dimethoxyquinazoline **5** with the commercially available 3-bromobenzaldehyde in the presence of 1,3-dimethylimidazolium iodide and sodium hydride in refluxing dioxane for 4 hours (Scheme 2) (32,33). The remaining 6,7-dimethoxyquinazoline derivatives were

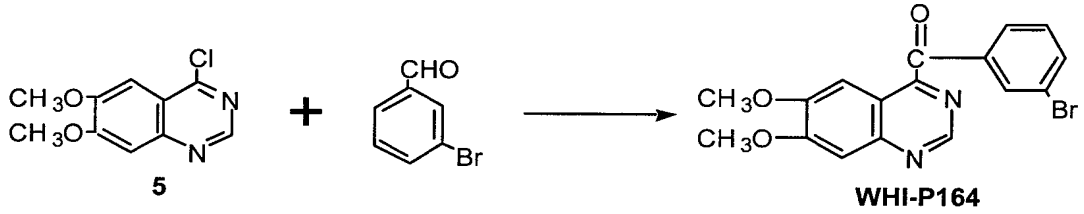
synthesized by condensing 4-chloro-6,7-dimethoxyquinazoline **5** with the corresponding substituted anilines as shown in Scheme 3.

Scheme 1. Synthesis of the common synthetic intermediate, 4-chloro-6,7-dimethoxyquinazoline.



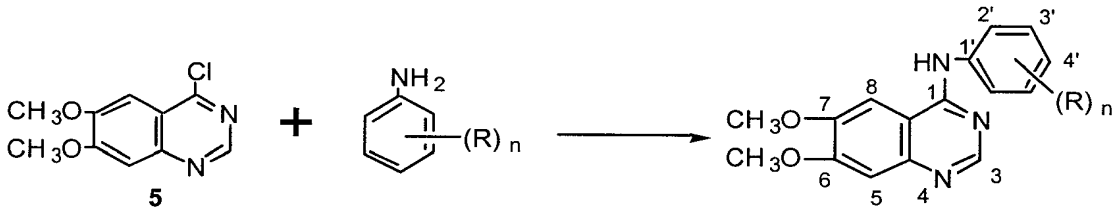
1 2 3 4 5

Scheme 2. Synthesis of WHI-P164.



5 WHI-P164

Scheme 3. Synthesis of WHI-P97.



5

WHI-P97 : 3'-Br, 4'-OH, 5'-Br
WHI-P111 : 3'-Br, 4'-CH_3
WHI-P131 : 4'-OH
WHI-P132 : 2'-OH
WHI-P154 : 3'-Br, 4'-OH
WHI-P197 : 3'-Cl, 4'-OH

Synthetic Procedures and characterization data: All chemicals were purchased from Aldrich (Milwaukee, WI) or Sigma (St. Louis, MO) and were used without further purification. Except where distinguished, each reaction vessel was secured with a rubber septa, and the reaction was performed under nitrogen atmosphere. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Mercury 300

instrument at ambient temperature in DMSO-d_6. Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. FT-IR spectra were recorded on a Nicolet Protege 460 spectrometer. GC/MS was obtained on a HP 6890 GC System equipped with a HP 5973 Mass Selective Detecter. TLC was performed on a precoated silica gel plate (Silica Gel KGF; Whitman Inc). Silica gel (200-400 mesh, Whitman Inc.) was used for all column chromatography separation.

4,5-Dimethoxy-2-nitrobenzamide **2**. A suspension of 4,5-dimethoxy-2-nitrobenzoic acid **1** (2 g; 8.8 mmol) in $SOCl_2$ (10 mL) was stirred under reflux for 50 minutes. After cooling, the reaction mixture was poured into a mixture of concentrated NH_4OH (50 mL) and ice (30 g). The precipitate were collected by filtration, washed with water, and dried to give 1.85 g crude crystals. After recrystallization from DMF, 1.76 g pure product was obtained (88.5%). ^1H NMR(DMSO-d_6) : δ 7.60(s, 2H, -NH_2), 7.57(s, 1H , 6-H), 7.12(s, 1H , 3-H), 3.90, 3.87(s, s, 6H, -OCH_3); IR(KBr) υ_{max}: 3454, 2840, 1670, 1512, 1274, 1227 cm^{-1}; GC/MS m/z 226(M^+, 10.0), 178(98.5), 163(100.0), 135(51.0).

6,7-Dimethoxyquinazoline-4(3H)-one **4**. $NaBH_4$ (400 mg) was added with stirring over 4 hours to a solution of 4,5-dimethoxy-2-nitrobenzamide **2** (1.58 g; 7 mmol) in MeOH containing catalytic amount of $CuSO_4$. The reaction mixture was poured into ice-water (200 mL) with stirring to give 4,5-dimethoxy-2-aminobenzamide **3** which was directly refluxed with HCOOH (20 mL) for 5 hours. After removal of solvent, the residue was recrystallized from DMF to give pure crystals **4** (1.18 g; 81.5 %). m.p. 295.0-297.0 °C; ^1H NMR (DMSO-d_6): δ 12.03 (br, s, 1H, -NH) ,7.99 (s, 1H, 2-H), 7.42 (s, 1H , 5-H), 7.11 (s, 1H , 8-H), 3.88, 3.85 (s, s, 6H, -OCH_3); IR(KBr)υ_{max}: 3015, 2840, 1648, 1504, 1261, 1070 cm^{-1}; GC/MS m/z 206(M^+, 100), 191(M^+ -CH_3 , 31.5), *163(16.7), 120(15.2).*

4-Chloro-6,7-dimethoxyquinazoline **5**. A suspension of 6,7-dimethoxyquinazoline-4(3H)-one **4** (12.36 g, 60 mmol) in $POCl_3$ (250 mL) was heated under reflux for 4 hr, when a clear solution was obtained. The $POCl_3$ was removed under reduced pressure, and the residue was dissolved in a mixture of CH_2Cl_2 and aqueous Na_2CO_3. The organic layer was dried and the solvent removed to give 4-chloro-6,7-dimethoxyquinazoline **5** (11.2 g, 83 %); m.p. 259.0-263.0 °C; ^1H

NMR (DMSO-d_6): d 8.75(s, 1H, 2-H), 7.53(s, 1H, 5-H), 7.25(s, 1H, 8H), 3.91(s, 3H, -OCH_3), 3.89(s, 3H, -OCH_3); IR (KBr) u_{max}: 2963, 2834, 1880, 1612, 1555, 1503, 1339, 1153, 962 cm^{-1}. GC/MS m/z 224(M^+, 100), 209(M^+ -CH_3 , 9.4), 189(19.39), (69(10.55).

4-(3'-Bromobenzoyl)-6,7-dimethoxyquinazoline **WHI-P164**. Sodium hydride (108 mg; 4.5 mmol) was added to a stirred solution of 4 -chloro-6,7-dimethoxyquinazoline **5** (896 mg; 4 mmol), 3-bromobenzaldehyde (832 mg; 4.5 mmol) and 1,3-dimethylimidazolium iodide (336 mg; 1.5 mmol) in dioxane (30 mL) and the mixture was refluxed for 4 hours. The reaction mixture was cooled to room temperature, poured into ice-water, and precipitate was collected. The yield was 81.2 % (1.05 g). m.p. 258.0-263.0 °C; ^{1}H NMR (DMSO-d_6): d 9.25 (s, 1H, 2-H), 8.14 (s, 1H, 5-H), 7.92-7.43 (m, 4H , 2', 4', 5', 6'-H), 7.40 (s, 1H, 8-H), 4.11 (s, 3H, -OCH_3), 4.00 (s, 3H, -OCH_3). IR (KBr) u_{max}: 3432, 1664, 1504, 1431, 1230 cm^{-1}. GC/MS m/z 374(M^+ +1, 48.96), 373(M^+, 34.93), 372(M^+-1,47.67), 357(58.74), 343(100.00), 293(M^+-Br, 31.48), 189(26.27).

General procedures for compounds synthesized according to **Scheme 3**. A mixture of 4-chloro-6,7-dimethoxyquinazoline **5** (448 mg; 2 mmol) and the appropriately substituted aniline (2.5 mmol) in 20 ml of alcohol (EtOH or MeOH) was heated to reflux. Heating was continued for 4-24 hours, sufficient Et_3N was added to neutralize the solution, and the solvent was then concentrated to give crude product, which was recrystallized from DMF.

4-(3',5'-Dibromo-4'-hydroxylphenyl)-amino-6,7-dimethoxyquinazoline **WHI-P97**: yield: 72.80%; m.p. >300.0 °C; ^{1}H NMR (DMSO-d6): δ 9.71 (s, 1H, -NH), 9.39 (s, 1H, -OH), 8.48 (s, 1H, 2-H), 8.07 (s, 2H, 2',6'-H), 7.76 (s, 1H, 5-H), 7.17 (s, 1H, 8-H), 3.94 (s, 3H, -OCH_3), 3.91 (s, 3H, -OCH_3); IR (KBr) υ_{max}: 3054 (br), 3419, 2868, 1627, 1512, 1425, 1250, 1155 cm-1; GC/MS m/z 456 (M^+ + 1. 54.40), 455 (M^+, 100.00), 454 (M^+ -1, 78.01), 439 (M^+ - OH, 7.96), 376 (M^+ +1 - Br, 9.76), 375 (M+ - Br, 10.91), 360 (5.23).

4-(3'-Bromo-4'-methylphenyl)-amino-6,7-dimethoxyquinazoline **WHI-P111**. yield: 82.22 %; m.p.225.0-228°C. ^{1}H NMR (DMSO-d_6): δ 10.23(s, 1H, -NH),

8.62(s, 1H, 2-H), 8.06(d, 1H, $J_{2',5'}$ = 2.1 Hz, 2'-H), 7.89(s, 1H, 5-H), 7.71(dd, 1H, $J_{5',6'}$ = 8.7 Hz, $J_{2',6'}$ = 2.1 Hz, , 6'-H), 7.37(d, 1H, $J_{5',6'}$ = 8.7 Hz, 5'-H), 7.21(s, 1H, 8-H), 3.96(s, 3H, $-OCH_3$), 3.93(s, 3H, $-OCH_3$). IR(KBr) υ_{max}: 3431, 3248, 2835, 1633, 1517, 1441, 1281, 1155 cm^{-1}. GC/MS m/z 375 (M^+ + 1 ,76.76), 374 (M^+,100.00), 373 (M^+ -1,76.91), 358 (M^+ + 1-OH, 11.15), 357(1.42), 356(6.31).

4-(4'-Hydroxylphenyl)-amino-6,7-dimethoxyquinazoline **WHI-P131**. yield: 84.29%; m.p. 245.0- 248.0 °C. ^1H NMR(DMSO-d_6): d 11.21(s, 1H, -NH), 9.70(s, 1H, -OH), 8.74(s, 1H, 2-H), 8.22(s, 1H, 5-H), 7.40(d, 2H, J = 8.9 Hz, 2',6'-H), 7.29(s, 1H, 8-H), 6.85(d, 2H, J = 8.9 Hz, 3',5'-H), 3.98(s, 3H, $-OCH_3$), 3.97(s, 3H, $-OCH_3$). IR(KBr) υ_{max}: 3428, 2836, 1635, 1516, 1443, 1234 cm^{-1}. GC/MS m/z 298 (M^++1, 100.00), 297(M^+, 26.56), 296(M^+-1, 12.46).

4-(2'-Hydroxylphenyl)-amino-6,7-dimethoxyquinazoline **WHI-P132**. yield: 82.49%; m.p. 255.0-258.0 °C; ^1H NMR(DMSO-d_6): d 9.78 (s, 1H, -NH), 9.29 (s, 1H, -OH), 8.33 (s, 1H, 2-H), 7.85 (s, 1H, 5-H), 7.41-6.83 (m, 4H, 3',4',5',6'-H), 7.16 (s, 1H, 8-H), 3.93 (s, 3H, $-OCH_3$), 3.92 (s, 3H, $-OCH_3$); IR (KBr) υ_{max}: 3500 (br), 3425, 2833, 1625, 1512, 1456, 1251, 1068 cm^{-1}; GC/MS m/z 298(M^+ +1,8.91), 297(M^+, 56.64), 281(M^+ +1-OH, 23.47), 280(M^+- OH, 100.00).

4-(3'-Bromo-4'-hydroxylphenyl)-amino-6,7-dimethoxyquinazoline **WHI-P154**. yield: 89.90%; m.p. 233.0-233.5 °C; ^1H NMR(DMSO-d_6): δ 10.08(s, 1H, -NH), 9.38(s, 1H , -OH), 8.40(s, 1H, 2-H), 7.89(d, 1H, $J_{2',5'}$ = 2.7 Hz, 2'-H), 7.75(s, 1H, 5-H), 7.55(dd, 1H, $J_{5',6'}$ = 9.0 Hz, $J_{2',6'}$ = 2.7 Hz, , 6'-H), 7.14(s, 1H, 8-H), 6.97(d, 1H, $J_{5',6'}$ = 9.0 Hz, 5'-H), 3.92(s, 3H, $-OCH_{ER}$), 3.90(s, 3H, $-OCH_3$); IR (KBr) υ_{max}: 3431(br), 2841, 1624, 1498, 1423, 1244 cm^{-1}; GC/MS m/z 378(M^+ +2, 90.68), 377(M^+ +1,37.49), 376(M^+,100.00), 360(M^+3.63), 298(18.86), 282 (6.65).

4-(3'-Chloro-4'-hydroxylphenyl)-amino-6,7-dimethoxyquinazoline **WHI-P197**. yield 84.14 %; m.p. 245.0 °C(dec); ^1H NMR(DMSO-d_6): δ 10.00 (s, 1H, -NH), 9.37 (s, 1H , -OH), 8.41 (s, 1H, 2-H), 7.78 (s, 1H, 5-H), 7.49 (d, 1H, $J_{2',5'}$ = 2.7 Hz, 2'-H), 7.55 (dd, 1H, $J_{5',6'}$ = 9.0 Hz, $J_{2',6'}$ = 2.7 Hz, , 6'-H), 7.16 (s, 1H, 8-H), 6.97 (d, 1H, $J_{5',6'}$ = 9.0 Hz, 5'-H), 3.93 (s, 3H, $-OCH_3$), 3.91 (s, 3H, $-OCH_3$); IR (KBr) υ_{max}: 3448, 2842, 1623, 1506, 1423, 1241 cm^{-1}; GC/MS m/z: 341(M^+ ,

100.00), 326(M^+-CH_3, 98.50), 310(M^+-OCH_3, 12.5), 295(9.0.), 189(13.5), 155(13.8).

Example 3
Lipid Lowering Effects of WHI-P164 in Hypercholesterolemic C57Bl/6 Mice.

A high cholesterol and high fat diet was used to induce hypercholesterolemia in C57Bl/6 mice, using the methods described by Paigen, et al. (Nishina, et al., 1993, *J. Lipid Res.* 34:1413-1422) and as described for Example 1. After two weeks, these mice exhibited a stable hypercholesterolemia with 4-times higher serum total cholesterol levels than control mice on regular rodent chow (451.4 ± 14.8, n=69 versus 117.8 ± 4.8, n=22, $p<0.0001$). Hypercholesterolemic mice treated with WHI-P164 for one week at 1.6mg/kg/day dose level exhibited a 23% reduction in mean serum total cholesterol levels (348.6 ± 27.6 mg/dL, n=9 versus 451.4 ± 14.8 mg/dL, n=69, p=0.02).

As shown by their serum lipoprotein profiles (Figure 1), hypercholesterolemic C57Bl/6 mice preferentially accumulated cholesterol rich VLDL (171 ± 19 mg/dL, n=4 versus 7 ± 4 mg/dL, n=3, p=0.0008) but no changes in HDL cholesterol levels (90 ± 9 mg/dL, n=4 versus 86 ± 13 mg/dL, n=3, p=0.8) (Table 2). WHI-P164 treatment caused a dramatic reduction of VLDL-C (Figure 1). In WHI-P164 treated mice, serum VLDL-C decreased immediately from 171 ± 19 mg/dL (n=4), to 99 ± 13 mg/dL (n=4) at the 1.6 mg/kg/day dose level (P=0.02) and further decreased to 78 ± 15 mg/dL (n=4) at the 16 mg/kg/day dose level (p=0.01) (Table 1). The calculated effective dose (ED_{50}) of WHI-P164 was 0.4 mg/kg/day with minimum serum VLDL-C level of 94 ± 9 mg/dL. Thus, WHI-P164 was only able to reduce VLDL-C by 45%. WHI-P164 treatment did not significantly affect serum HDL-C levels (Figure 1 and Table 1), even though there was a trend for decreasing HDL-C with increasing WHI-P164 dose levels.

Table 1. Reduction of VLDL-C by WHI-P164

	Dose (mg/kg)	N	VLDL-C (mg/dL)	p	HDL-C (mg/dL)	p
apo $e^{+/+}$ (C57B1/6)						
Rodent Chow	0.0	3	7±4	--	86±13	--
Cocoa Butter Diet	0.0	4	171±19	--	90±9	--
	1.6	4	99±13	0.02	93±8	0.8
	4.0	4	106±15	0.04	86±5	0.7
	8.0	3	101±7	0.03	81±9	0.5
	16.0	4	78±15	0.01	74±10	0.3
apo $e^{-/-}$(C57B1/6)	0.0	4	516±158	--	21±3	--
Rodent Chow	8.0	8	190±13	0.01	50±10	0.09
Western diet	0.0	3	1296±118**	--	50±9	--
	40.0	2	587±4	0.02	90±4	0.04

Plasma VLDL-C and HDL-C were calculated based on the lipoprotein profiles. Treated and untreated groups were compared by student t-test.
*P-values refer to differences between vehicle-treated and WHI-P164 treated mice.
**P=0.01 for the difference of VLDL-C levels of *apo* $e^{-/-}$ via the rodent chos vs. western diet (1296±118 mg/dL vs. 516±158 mg/dL.

WHI-P164 treatment at the indicated dose levels, which were much lower than the highest nontoxic dose of 2 kg/kg, was not associated with any obvious clinical or laboratory signs of toxicity. In particular, liver enzymes (ALT, ALP, LDH) and BUN/Creatinine remained within normal limits (Table 2). Histopathologic examination of tissues did not reveal any drug related toxic lesions.

Table 2

Blood Chemistry of Treated C57B1/6 Mice

	1 week		2 weeks	
	+P164	-P164	+P164	-P164
Net weight change (g)	-0.26 ± 0.22 (n=17)	0.87 ± 0.33 (n=10)	1.88 ± 0.42 (n=5)	3.07 ± 0.50 (n=10)
ALT (IU/L)	---	---	148 ± 54 (n=5)	177 ± 19 (n=10)
ALP (IU/L)	101 ± 3 (n=5)	110 ± 9 (n=5)	94 ± 10 (n=5)	132 ± 6 (n=10)
LD-L (IU/L)	1402 ± 144 (n=5)	1533 ± 189 (n=5)	459 ± 83 (n=5)	426 ± 42 (n=9)
BUN (mg/dL)	25.4 ± 2.0 (n=5)	27.6 ± 2.0 (n=5)	---	---
Glucose (mg/dL)	201 ± 18 (n=25)	144 ± 9 (n=24)		

Example 4
Lipid Lowering Effects of WHI-P164 in
Hypercholesterolemic Apo E-Deficient Mice

ApoE, a component of hepatic VLDL and intestinal chylomicron, controls the catabolism of these particles by serving as a ligand for LDL receptor and LDL-like receptor. Functionally defective *apo e* mutation is associated with type III dyslipoproteinemia, a condition mimicked by *apo* $e^{-/-}$ mice. As *apo* $e^{-/-}$ mice exhibited hypercholesterolemia even in the absence of dietary cholesterol supplementation due to a delayed clearance of hepatic VLDL particles, WHI-P164 was analyzed for its ability to reduce VLDL-C.

On regular rodent chow, the serum total cholesterol level of *apo* $e^{-/-}$ mice was 836 ± 57 mg/dL (n=47). After one week therapy with 8 mg/kg/day WHI-P164 (n=12), the mean serum total cholesterol level was reduced to 551 ± 29 mg/dL (p=0.02). Even in the absence of dietary cholesterol challenge, these *apo* $e^{-/-}$ mice exhibited higher VLDL-C (516 ± 158 mg/dL, n=4 versus 7 ± 4 mg/dL, n=3, p=0.04) and lower HDL-C levels than *apo* $e^{+/+}$ C57Bl/6 mice on rodent chow (21 ± 3 mg/dL, n=4 versus 86 ± 13 mg/dL, n=3, p=0.0024) (Table 1). After one week therapy with 8 mg/kg/day WHI-P164, VLDL-C was decreased from 516 ± 158 mg/dL, n=4, to 190 ± 13 mg/dL, n=8, p=0.01 and HDL-C was slightly increased from 21 ± 3 mg/dL, n=4, to 50 ± 10 mg/dL (Table 1, Figure 2).

When challenged with a cholesterol-rich diet, these animals developed frank hypercholesterolemia due to delayed clearance of intestinal chylomicrons which shuttle the dietary cholesterol from the intestine to the liver. In the present study, all of the 46 ApoE-deficient mice that were fed Western diet (14) became severely hypercholesterolemic with a mean serum total cholesterol level of 1491 ± 59 mg/dL. One week WHI-P164 therapy reduced the mean serum total cholesterol level of hypercholesterolemic *apo* $e^{-/-}$ mice to 1194 ± 84 mg/dL (p=0.006) at a dose level of 8 mg/kg/day (n=19) and to 876 ± 57 mg/dL (p<0.0001) at a dose level of 40 mg/kg/day (n=17) (Table 3). As shown in Table 1, dietary cholesterol challenge increased VLDL-C levels from 516 ± 158 mg/dL, (n=3) to

1296 ± 118 mg/dL (n=3), p=0.01. After one week of therapy with 40 mg/kg/day WHI-P164, VLDL-C was decreased from 1296 ± 118 mg/dL, n=3, to 587 ± 4 mg/dL, n=2, p=0.02 and HDL-C was slightly increased from 50 ± 9 mg/dL, n=3, to 90 ± 4 mg/dL, n=2, p=0.04 (Table 1, Figure 3). In contrast to WHI-P164, other dimethoxyquinazoline derivatives did not exhibit cholesterol-lowering activity in C57B1/6 or *apo* $e^{-/-}$ mice (Table 4).

Table 3.
Structure Function Evaluation of WHI-P164
and Other Dimethoxyquinazoline Derivatives

Compounds	[a]*apo* $e^{-/-}$(C57B1/6)		[b]*apo* $e^{+/+}$(C57B1/6)	
	N	Cholesterol (mg/dL)	N	Cholesterol (mg/dL)
Vehicle	46	1491±59	69	451±15
WHI-P97	5	1129±46	10	413±18
WHI-P111	5	1425±92	4	406±11
WHI-P131	4	1532±77	5	433±20
WHI-P132	5	1774±61	--	ND
WHI-P154	5	1492±110	--	ND
WHI-P164	17	876±57*	9	349±28**
WHI-P197	5	1621±146	5	372±20

[a]*apo* $e^{-/-}$(C57B1/6) mice were placed on Western diet for two weeks, followed by treatment with the drug for 1 week at 40 mg/kg/day.
[b]*apo* $e^{+/+}$(C57B1/6) mice were placed on Cocoa butter diet for two weeks, followed by treatment with the drug for 1 week at 1.6 mg/kg/day.
*$p<0.0001$ **$p=0.02$.

WHI-P164 therapy, at either 8 mg/kg/day or 40 mg/kg/day, was not associated with any obvious clinical or laboratory signs of toxicity. In particular, liver enzymes (ALT, AST, ALP, LDH), CPK levels, and BUN/Creatinine remained within normal limits. Histopathologic examination of tissues did not reveal any drug related toxic lesions.

Example 5
Effects of WHI-P164 on Triglyceride (TG) Synthesis

apo $e^{-/-}$ mice accumulate large amounts of TG-rich lipid droplets in their livers, due a blocked VLDL-TG secretion (Kuipers et al., 1997, *J. Clin. Invest.*

100:2915-2922). Treatment with 40 mg/kg/day WHI-P164 for 1 week signicantly reduced the Oil Red O stainable lipid material in the livers of Apo E-deficient mice that were fed on a Western diet (Figures 4A-4D). Image analysis demonstrated a 75% reduction of lipid material in treated mice (658 ± 256 red pixels/field, n=11 versus 2590 ± 401 red pixels/field, n=12; p=0.0007). When tissue lipids were measured, there was a 22% reduction in hepatic cholesterol 39% reduction in hepatic triglycerides. As shown in Table 4, the reduction in hepatic TG accumulation was not due to depletion of precursors for hepatic TG accumulation, namely plasma free fatty acid (FFA) and triglyceride.

Table 4
Analysis of Tissue Lipids

Lipid Levels	WHI-P164	Control (vehicle)	p Value
hepatic cholesterol (mg/g wet wt)	51.4±4.4 (n=7)	65.9±5 (n=8)	p=0.5
hepatic triglycerides (mg/g wet wt)	211.6±27 (n=7)	349.3±34 (n=8)	p=0.008
plasma FFA (mM)	3.6±0.3 (n=12)	3.2±0.3 (n=11)	
plasma TG (mg/dL)	165±19 (n=15)	167±14 (n=17)	

Example 6
Regression of Pre-existing Atherosclerotic Lesions

The effectiveness of WHI-P164 against pre-existing atherosclerotic lesions in *apo* $e^{-/-}$ mice kept 7 months on high fat Western diet was examined. *apo* $e^{-/-}$ mice were treated with either vehicle control or WHI-P164 at 40mg/kg/day for one month. As shown in Figure 6A, before treatment, fatty streaks stainable by Sudan IV covered 40 ± 5 % (n=3) of the aortic surface of these mice. Treatment with WHI-P164 caused a marked regression of the atherosclerotic lesions with fatty streaks covering only 23 ± 2 % (n=7) of the aortic surface (Figure 6B), as compared to 40 ± 5 % in untreated mice (n=3) (Figure 6A) (p= 0.002) and 40 ± 5 % in the vehicle-treated mice (n=8) (Figure 6C) (p=0.007).

Example 7
Effect of WHI-P164 on Liver Lipoprotein Synthesis Determination of In Vivo Hepatic VLDL-TG Production Using Triton WR1339

To determine if WHI-P164 prevent hepatic TG accumulation by relieving 9 block in VLDL-TG secretion or by inhibiting upstream TG synthesis, *in vivo* hepatic VLDL-TG synthesis was analyzed. Synthesis was determined by following the accumulation of VLDL-TG after shutting down VLDL catabolism with Triton WR1339 injection and intestinal chylomicron synthesis with a 36 hours fast.

C57Bl/6 and apoE-deficient mice were treated with intra peritoneal injections of vehicle or 40 mg/kg WHI-P164 (in 50 µl vehicle) for 7 consecutive days, followed by a 36 hour fast to shut down intestinal chylomicron synthesis. Subsequently, mice were injected intraperitonealy (i.p.) with 500 mg/kg Triton WR1339 (Sigma Chemical Co.) in 0.9% NaCl to completely inhibit their plasma VLDL clearance, as previously reported (Kuipers et al., 1996, *Heptology* 24:241-247). Blood samples (50 µl) were taken at 0 and 4 hours after Triton WR1339 injection and plasma TG was measured enzymatically using a commercially available enzymatic kit (Sigma Chemical Co.), as previously reported (Kuipers, *supra*).

WHI-P164 treated *apo* $e^{-/-}$ mice exhibited significantly less accumulation of VLDL-TG than vehicle-treated control mice after Triton WR1339 treatment ($p=0.0002$), although they exhibited similar pre-Triton WR1339 serum triglyceride levels (Table 5). WHI-P164 treated C57Bl/6 mice also exhibited significantly less accumulation of VLDL-TG than vehicle-treated control mice ($p=0.003$), although they exhibited similar pre-Triton WR1339 serum triglyceride levels (Table 5). The data support the hypothesis that WHI-P164 inhibited TG synthesis, thereby preventing hepatic accumulation of TG in *apo* $e^{-/-}$ mice.

Table 5
Inhibition of Hepatic VLDL-TG Synthesis by WHI-P164

	Triglyceride (mg/dL)	
	Pre-Triton WR 1339	Post-Triton WR 1339
apo $e^{-/-}$(C57B1/6)		
Vehicle	110±12 (n=8)	744±37 (n=14)
WHI-P164	155±14 (n=10)	465±46 (n=8)*
apo $e^{+/+}$(C57B1/6)		
Vehicle	143±6 (n=5)	1354±44 (n=12)
WHI-P164	124±12 (n=5)	1031±95 (n=8)**

Hepatic VLDL-TG was determined as accumulation of triglyceride after injection of Triton WR1339, which shuts down VLDL catabolism, and 36 hours fast which shuts down intestinal chylomicron synthesis. Student t-test was used to compare the WHI-164 treated group versus the vehicle treated group.
*p=0.0002 **p=0.003

The decrease in TG synthesis was not specific to the liver, as intestinal chylomicron-TG was also inhibited by WHI-P164 therapy. When 36 hours fasted mice were fed again, they immediately consumed the food and exhibited a time dependent accumulation in plasma TG, due to intestinal chylomicron-TG synthesis. Postprandial accumulation was inhibited by WHI-P164 therapy (Figure 5). Inhibition reached statistical significance at 6 hours in vehicle treated mice having a plasma TG level of 272 ± 25 mg/dL, n=5, as compared to a plasma TG level of 155 ± 32 mg/dL, n=5, p= 0.004 in WHI-P164 treated mice.

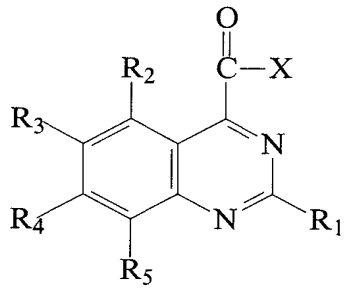
The present invention should not be considered limited to the particular examples described above, but rather should be understood to cover all aspects of the invention as fairly set out in the attached claims. Various modifications, equivalent processes, as well as numerous structures to which the present invention may be applicable will be readily apparent to those of skill in the art to which the present invention is directed upon review of the instant specification.

All publications and patent applications in this specification are indicative of the level of ordinary skill in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if

each individual publication or patent application was specifically and individually indicated by reference.

WE CLAIM:

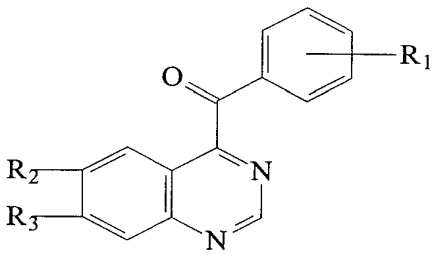
1. A compound comprising the formula:



wherein X comprises a straight or branched chain or cyclic alkyl, or an aromatic ring structure, and wherein each of R_1 - R_5 are independently selected from H, OH, NH_2, SH, alkyl, alkoxy, and acyloxy.

2. The compound of claim 1, wherein the straight or branched chain or cyclic alkyl, or an aromatic ring structure includes one or more substitutions selected from halo, OH, NH_2, SH, alkyl, alkoxy, and acyloxy .

3. The compound of claim 1 comprising the formula:



wherein each of R_1- R_3 independently comprise H, OH, alkoxy, acyloxy, SH, NH_2 or halo.

4. The compound of claim 1, wherein said compound is 4-(3'-bromobenzoyl)6,7-dimethoxy quinazoline (WHI-P164).

5. A composition comprising the compound of claim 1 and a carrier.

6. The composition of claim 5, comprising 4-(3'-bromobenzoyl)6,7-dimethoxy quinazoline (WHI-P164).

7. A method for reducing blood cholesterol in a subject comprising administering to the subject an effective cholesterol-lowering amount of the compound of claim 1.

8. The method of claim 7, wherein the compound of claim 1 is 4-(3'-bromobenzoyl)6,7-dimethoxy quinazoline (WHI-P164).

9 The method of claim 7, wherein said compound is co-administered with an inhibitor of HMG CoA reductase.

10. The method of claim 7, wherein said compound is administered at a daily dose of from about 50 to about 500 μg/ day.

11. A dietary supplement comprising the compound of claim 1.

12. A cholesterol-lowering composition comprising an effective cholesterol lowering amount of the compound of claim 1.

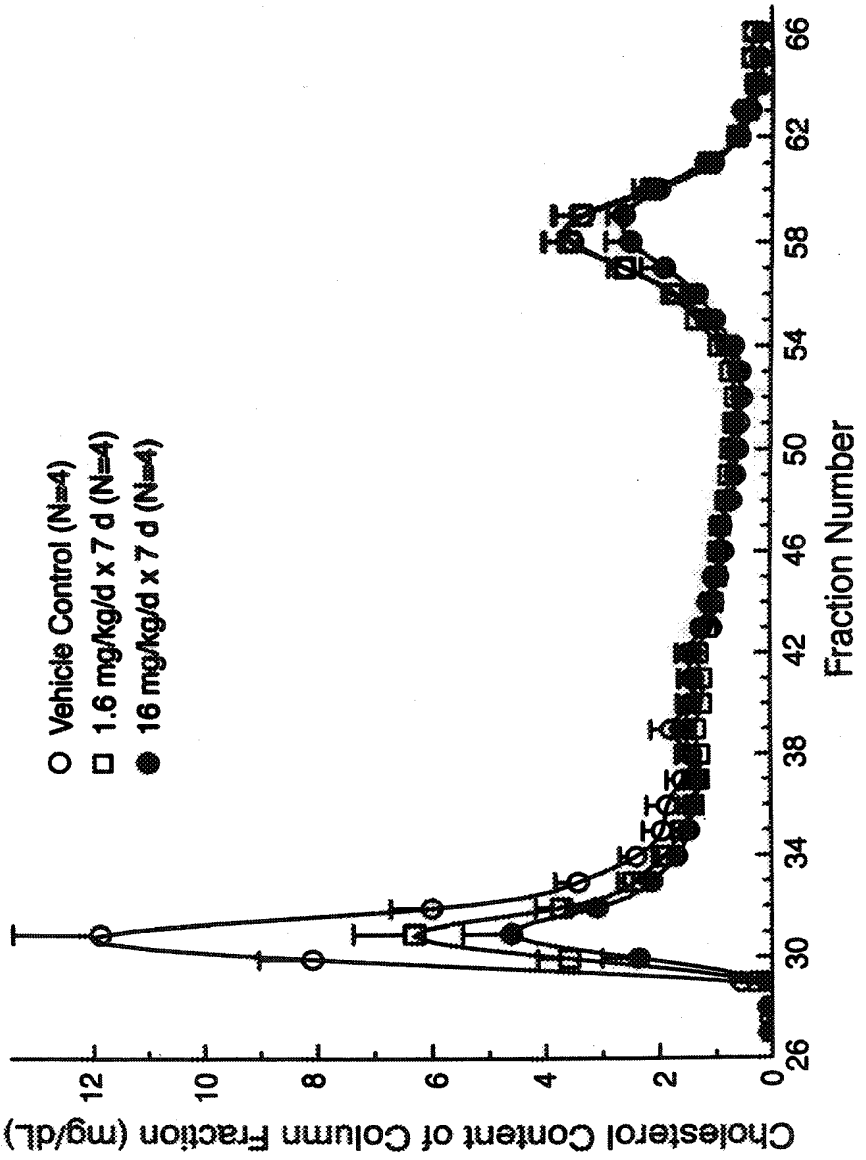

Vehicle Control (N=4)
1.6 mg/kg/d x 7 d (N=4)
16 mg/kg/d x 7 d (N=4)
Cholesterol Content of Column Fraction (mg/dL)
Fraction Number
0
2
4
6
8
10
12
26
30
34
38
42
46
50
54
58
62
66

FIG. 1

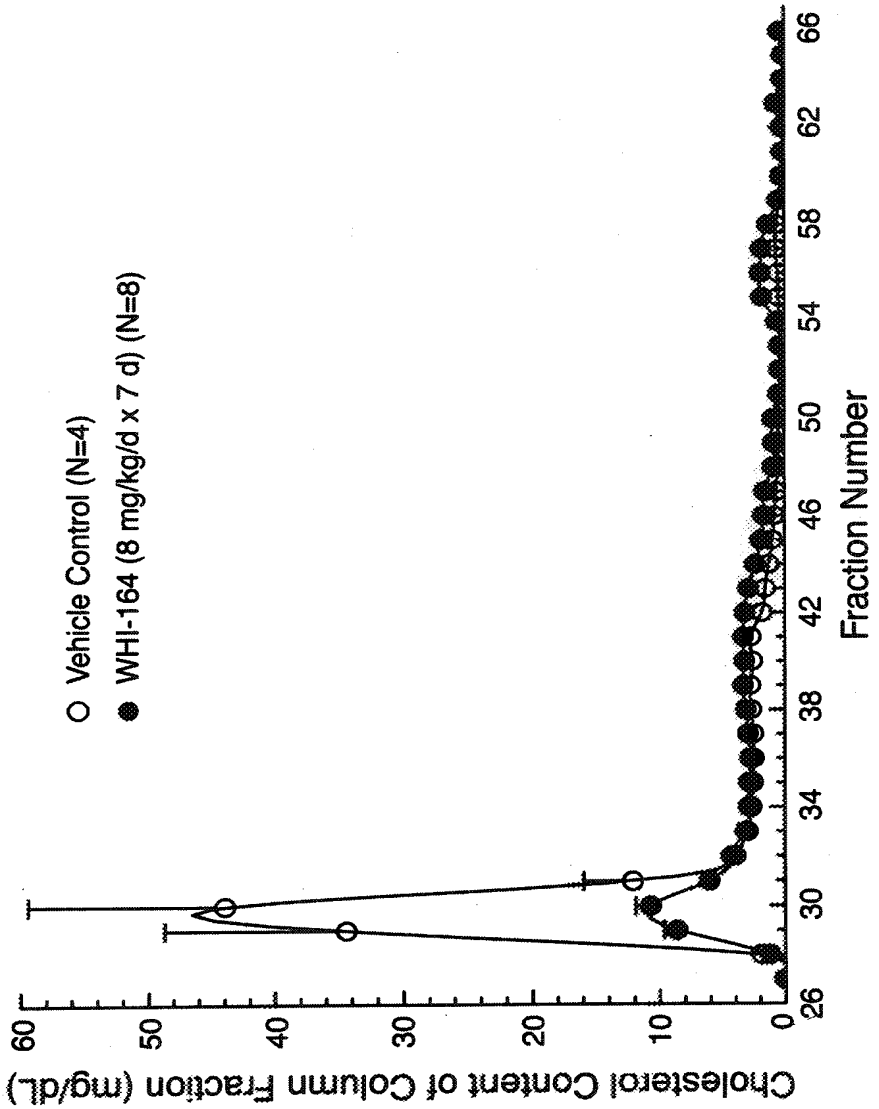

Cholesterol Content of Column Fraction (mg/dL)
0
10
20
30
40
50
60
26
30
34
38
42
46
50
54
58
62
66
Fraction Number
○ Vehicle Control (N=4)
● WHI-164 (8 mg/kg/d x 7 d) (N=8)

FIG. 2

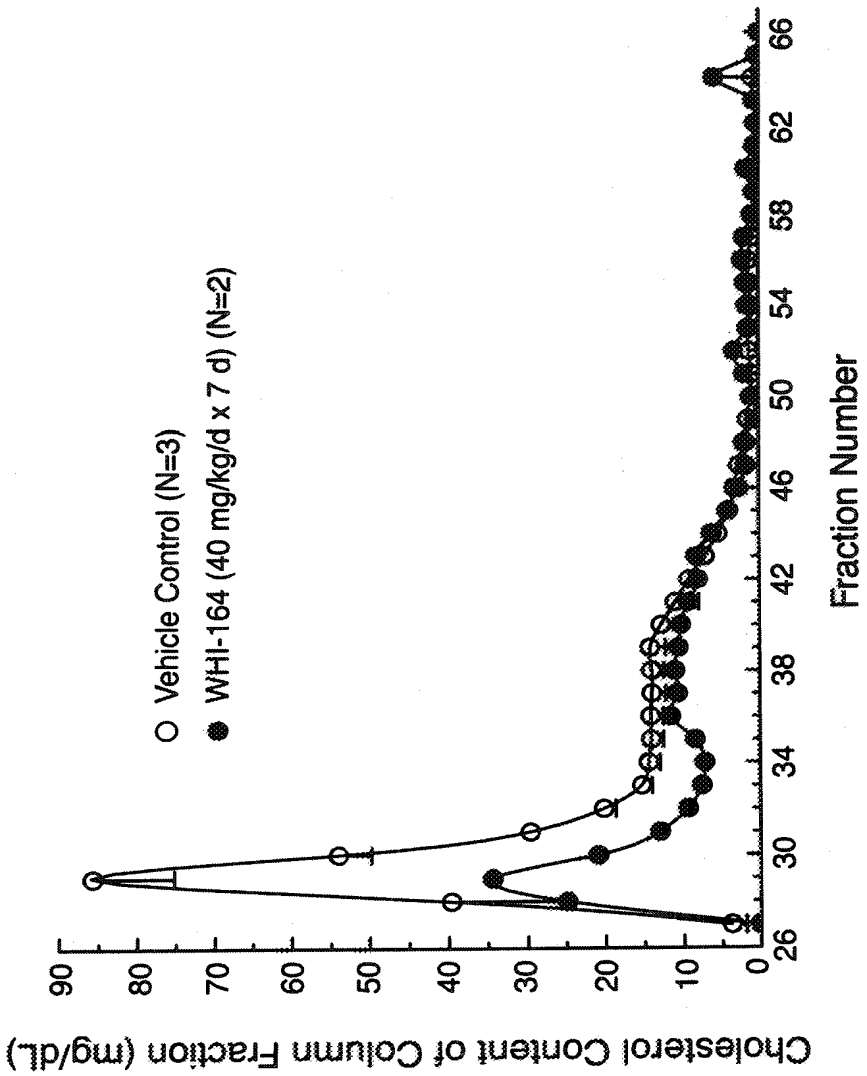

Cholesterol Content of Column Fraction (mg/dL)
Fraction Number
O Vehicle Control (N=3)
● WHI-164 (40 mg/kg/d x 7 d) (N=2)
0
10
20
30
40
50
60
70
80
90
26
30
34
38
42
46
50
54
58
62
66

FIG. 3

Fig. 4B

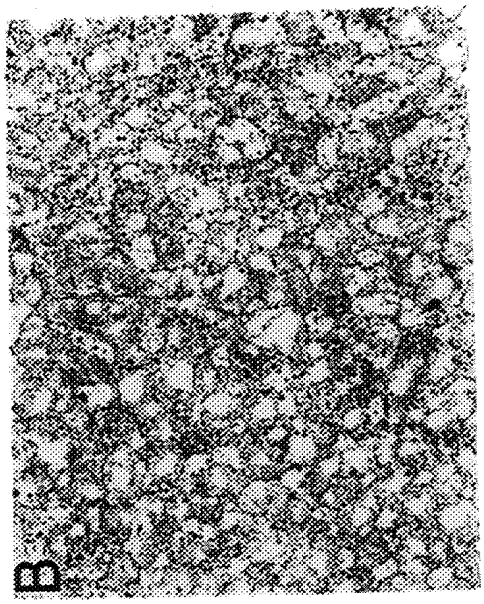


Fig. 4D

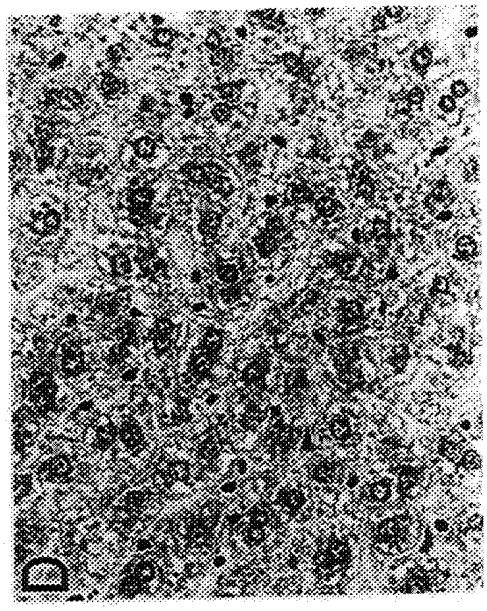


Fig. 4A

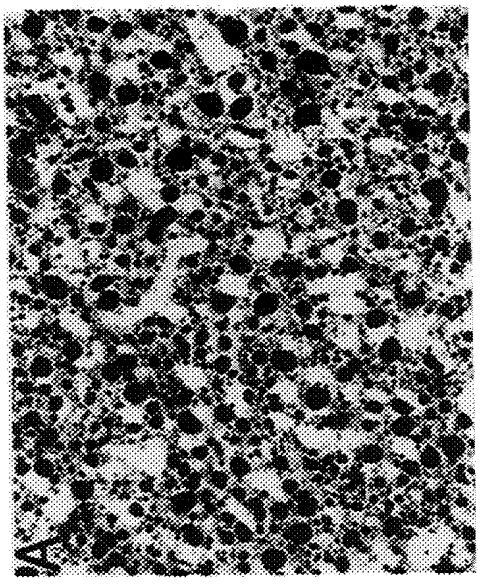
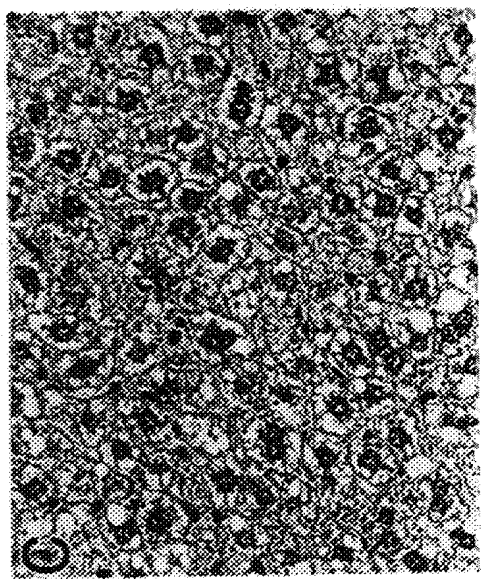


Fig. 4C



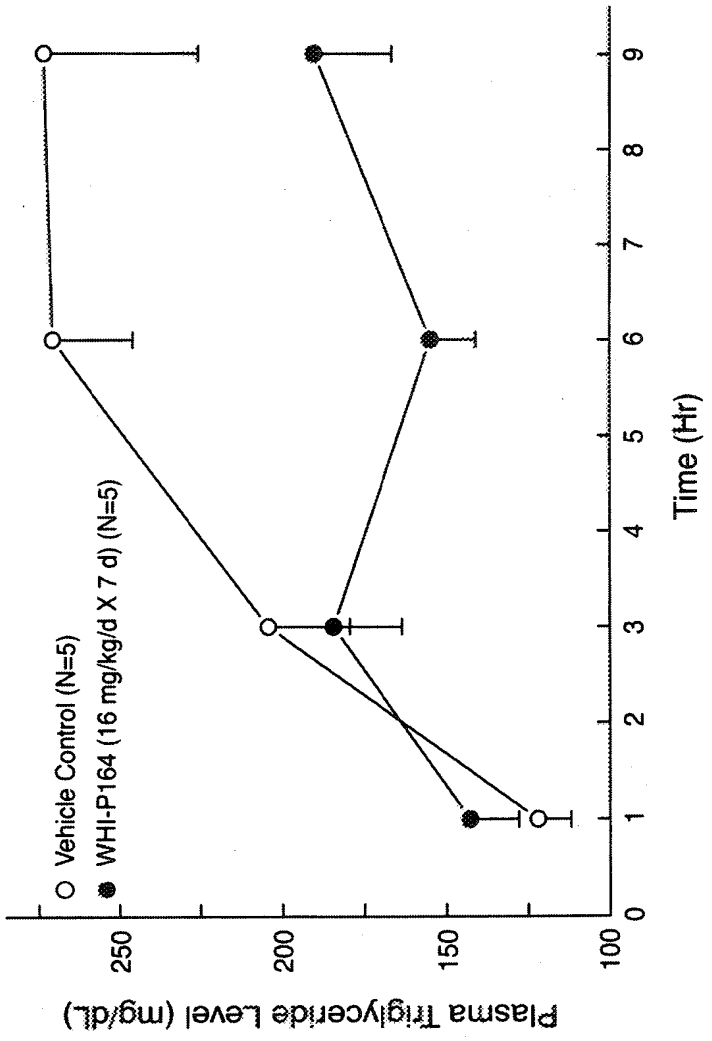

Plasma Triglyceride Level (mg/dL)
250
200
150
100
0
1
2
3
4
5
6
7
8
9
Time (Hr)
Vehicle Control (N=5)
WHI-P164 (16 mg/kg/d X 7 d) (N=5)

FIG. 5

Fig. 6A

A. Untreated

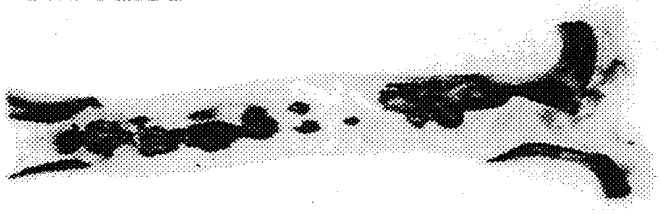


Fig. 6B

B. WHI-P164 x 1 mo

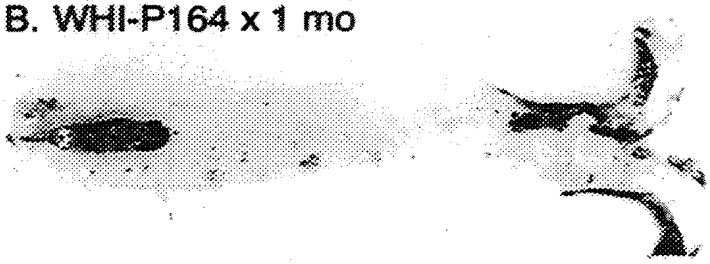


Fig. 6C

C. Vehicle x 1 mo

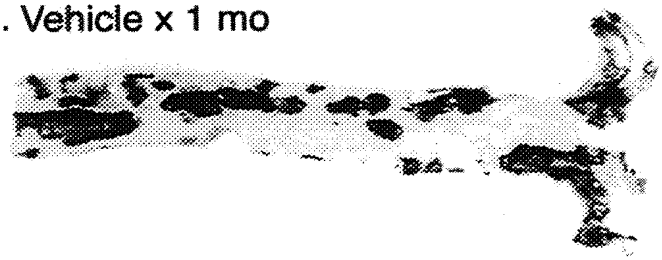
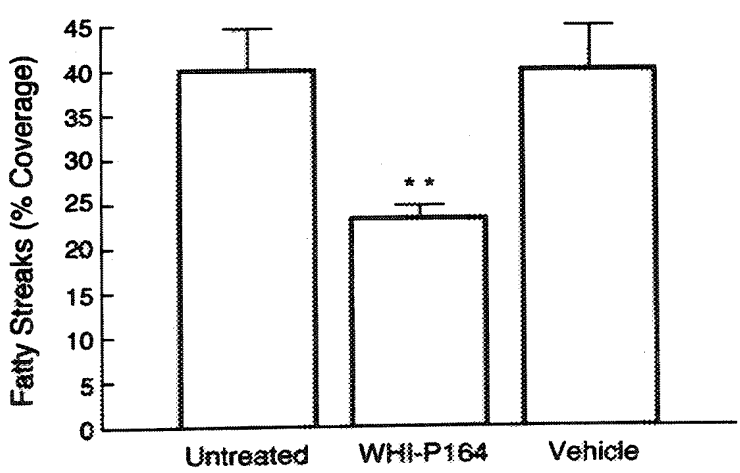


Fig. 6D

D.



INTERNATIONAL SEARCH REPORT

Intern ıal Application No
PCT/US 99/15841

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D239/74 A61K31/517

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Y. SUZUKI ET AL.: "CARBON-CARBON BOND CLEAVAGE" CHEMICAL AND PHARMACEUTICAL BULLETIN., vol. 46, no. 2, February 1998 (1998-02), pages 199-206, XP002120570 PHARMACEUTICAL SOCIETY OF JAPAN. TOKYO., JP ISSN: 0009-2363 page 199 -page 202; examples 34,36 --- -/--	1,2

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 October 1999	10/11/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Francois, J

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	A.MIYASHITA,Y. SUZUKI ET AL.: "CATALYTIC ACTION OF AZOLIUM SALTS.IV." CHEMICAL AND PHARMACEUTICAL BULLETIN., vol. 42, no. 10, October 1994 (1994-10), pages 2017-2022, XP002120571 PHARMACEUTICAL SOCIETY OF JAPAN. TOKYO., JP ISSN: 0009-2363 page 2017 -page 2019 ---	1,2
X	A.MIYASHITA ET AL.: "CATALYTIC ACTION OF AZOLIUM SALTS.II." CHEMICAL AND PHARMACEUTICAL BULLETIN., vol. 40, no. 1, January 1992 (1992-01), pages 43-48, XP002120572 PHARMACEUTICAL SOCIETY OF JAPAN. TOKYO., JP ISSN: 0009-2363 page 43 -page 48 ---	1,2
X	A. MIYASHITA ET AL.: "AN APPROACH TO THE SYNTHESIS OF A PAPAVERINE ANALOGUE" HETEROCYCLES., vol. 40, no. 2, 1995, pages 653-660, XP002120573 ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM., NL ISSN: 0385-5414 page 653 -page 659 ---	1,2
X	M. LEMPERT-SRETER ET AL.: "ELECTRON DEFICIENT HETEROAROMATIC AMMONIOAMIDATES.PART 26." JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS 1., no. 6, 1984, pages 1143-1151, XP002120574 CHEMICAL SOCIETY. LETCHWORTH., GB ISSN: 0300-922X page 1143 -page 1147; example 13B ---	1,2
X	CHEMICAL ABSTRACTS, vol. 85, no. 7, 1976 Columbus, Ohio, US; abstract no. 46568z, HIGASHINO,TAKEO ET AL.: "STUDIES ON THE REACTION OF QUINAZOLINE WITH AROMATIC ALDEHYDES" page 530; column 1; XP002120575 abstract & YAKUGAKU ZASSHI, vol. 96, no. 4, 1976, pages 397-400, JAPAN --- -/--	1,2

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INTERNATIONAL SEARCH REPORT

Intern ıal Application No
PCT/US 99/15841

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 80, no. 26, 1974 Columbus, Ohio, US; abstract no. 47718g, E.C. TAYLOR ET AL.: "GEN. PROCEDURE FOR THE SYNTHESIS OF EPOXYALKYLATED AND ACYLATED HETEROCYCLES." page 337; XP002120576 abstract & HETEROCYCLES., vol. 1, no. 1-2, 1973, pages 59-65, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM., NL ISSN: 0385-5414	1
A	WO 95 15758 A (RHONE-POULENC) 15 June 1995 (1995-06-15) page 1 -page 25; claims	1,5,11

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INTERNATIONAL SEARCH REPORT

In ational application No.
PCT/US 99/ 15841

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 7-10
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 7 to 10 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Intern ...al Application No
PCT/US 99/15841

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9515758 A	15-06-1995	US 5480883 A	02-01-1996
		US 5710158 A	20-01-1998
		AU 1305095 A	27-06-1995
		EP 0871448 A	21-10-1998
		SG 54172 A	16-11-1998
		US 5795889 A	18-08-1998
		US 5646153 A	08-07-1997
		US 5721237 A	24-02-1998
		US 5714493 A	03-02-1998
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